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Changes induced by malathion, methylparathion and parathion on membrane lipid physicochemical properties correlate with their toxicity

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Abstract

Perturbations induced by malathion, methylparathion and parathion on the physicochemical properties of dipalmitoylphosphatidylcholine (DPPC) were studied by fluorescence anisotropy of DPH and DPH-PA and by differential scanning calorimetry (DSC). Methylparathion and parathion (50 μM) increased the fluorescence anisotropy evaluated by DPH-PA and DPH, either in gel or in the fluid phase of DPPC bilayers, but mainly in the fluid phase. Parathion is more effective than methylparathion. On the other hand, malathion had almost no effect. All the three xenobiotics displaced the phase transition midpoint to lower temperature values and broadened the phase transition profile of DPPC, the effectiveness following the sequence: parathion > methylparathion ≫ malathion. A shifting and broadening of the phase transition was also observed by DSC. Furthermore, at methylparathion/lipid molar ratio of 1/2 and at parathion/lipid molar ratio of 1/7, the DSC thermograms displayed a shoulder in the main peak, in the low temperature side, suggesting coexistence of phases. For higher ratios, the phase transition profile becomes sharp as the control transition, but the midpoint is shifted to the previous shoulder position. Conversely to methylparathion and parathion, malathion did not promote phase separation. The overall data from fluorescence anisotropy and calorimetry indicate that the degree of effect of the insecticides on the physicochemical membrane properties correlates with toxicity to mammals. Therefore, the in vivo effects of organophosphorus compounds may be in part related with their ability to perturb the phospholipid bilayer structure, whose integrity is essential for normal cell function.

Keywords: Organophosphorus compounds; Physicochemical properties of lipids; Fluorescence anisotropy; Differential scanning calorimetry

1. Introduction

Organophosphorus insecticides are widely used, for agricultural and domestic purposes, in the control of insect pests. However, most of the above compounds are highly toxic to non-target organisms, either invertebrates or vertebrates, including humans [1,2]. Therefore, it is of major interest to identify the molecular mechanisms of action that can help in the development of insecticides with improved selectivity.

The acute toxicity of organophosphorus insecticides is predominantly mediated by the inhibition of acetylcholinesterase [3]. However, a current understanding of how these compounds interact with the acetylcholinesterase is incomplete, since the capacity

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to phosphorylate the enzyme appears to decrease as the organophosphorus concentration increases [4]. On the other hand, the chronic toxicity resulting from continuous exposure to these compounds [5,6] escapes a rational understanding on the basis of molecular mechanisms.

The lipophilicity of organophosphorus insecticides favors their incorporation in membranes [7,8]. The incorporated compounds elicit physical and chemical perturbations and presumably dysfunction of the membranes. In previous work of our own [9–12] and of others [13–16] perturbations of basic membrane mechanisms have been identified. A correlation between the perturbations in membrane basic mechanisms and insecticide toxicity is often apparent. On the other hand, evidence is accumulating that lipid bilayer structure and dynamics are implicated in membrane functionality [17–20]. Therefore, the primary effects of insecticides may result from physicochemical changes at the level of membrane lipid structure and organization. Consequently, the aim of the present work is to further elucidate a putative relationship between changes in the physicochemical properties of the membrane and insecticide toxicity, attempting to clarify the molecular mechanisms underlying the toxic effects of insecticides. Thus, changes in the physicochemical properties of model dipalmitoylphosphatidylcholine (DPPC) bilayers induced by malathion, methylparathion and parathion, evaluated by differential scanning calorimetry (DSC) and by fluorescence anisotropy of 1,6-diphenyl-1,3,5-hexatriene (DPH) and [*p*-(6-phenyl)-1,3,5-hexatrienyl] phenylpropionic acid (DPH-PA) have been studied.

2. Materials and methods

2.1. Materials

DPPC was obtained from Sigma Chemical Co., St. Louis, MO, USA. The probes DPH and DPH-PA were purchased from Molecular Probes, Inc., Eugene, OR, USA. The insecticides malathion, *O*,*O*-dimethyl *S*-(1,2-dicarboxyethyl) phosphorodithioate, methylparathion, *O*,*O*-dimethyl-*O*-(*p*-nitrophenyl) phosphorothioate and parathion, *O*,*O*-diethyl-*O*-(*p*-nitrophenyl) phosphorothioate (Fig. 1) were supplied by Supelco, Inc., Bellefonte, PA, USA. All the compounds were of the highest commercially available purity.

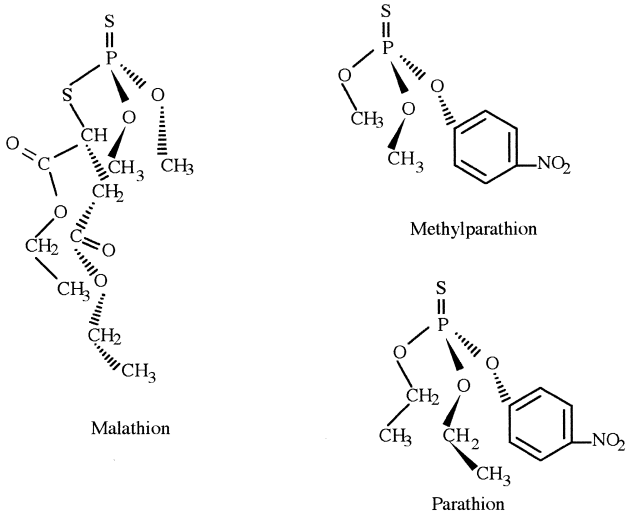

Fig. 1. Structure of malathion, *O*,*O*-dimethyl *S*-(1,2-dicarboxyethyl) phosphorodithioate, methylparathion, *O*,*O*-dimethyl-*O*-(*p*-nitrophenyl) phosphorothioate, and parathion, *O*,*O*-diethyl-*O*-(*p*-nitrophenyl) phosphorothioate.

2.2. Methods

2.2.1. Preparation of membranes for fluorescence anisotropy studies

Model membranes were prepared as described elsewhere [21]. Briefly, solutions of pure DPPC in $CHCl_3$ were evaporated to dryness in round-bottomed flasks. The resulting lipid film on the wall of the round-bottomed flask was hydrated with an appropriate volume of 50 mM KCl, 10 mM Tris–maleate (pH 7.0) and dispersed under N_2 atmosphere by handshaking in a water bath set 7–10°C above the transition temperature of DPPC and multilamellar vesicles were obtained. The final nominal concentration of the lipid was 345 μM. The model membranes were briefly sonicated in a low-energy water sonifier to disperse aggregates, in order to decrease the light scattering and to improve the readings of fluorescence. Sonication was applied for a controlled period of time to avoid the turbidity decreasing below 0.15 absorbance units at 600 nm.

2.2.2. Incorporation of the probes and insecticides into membranes

Few μl of the fluorescence probes in dimethylform-

amide were injected into membrane suspensions (345 μM in lipids) to give a final lipid/probe molar ratio of about 300/1. The mixture was initially vigorously vortexed for 10 s and then the insecticides were added from concentrated ethanolic solutions. The mixture was incubated in the dark to protect the probes, for a period of 18–20 h, i.e. the time required for equilibrium, since the insecticides have to penetrate multiple lipid bilayers. The incubation was performed at the phase transition temperature of DPPC, at which the incorporation of the insecticides is maximal [7]. Control samples received equivalent volumes of dimethylformamide and ethanol. Added solvents (few μl) had no detectable effects on the assays.

2.2.3. Fluorescence anisotropy measurements

Fluorescence spectra were recorded in a Perkin-Elmer spectrofluorometer, model MPF-3, provided with a thermostated cell holder. The excitation was set a 365 nm and the emission was detected at 450 nm. The excitation and emission slits were 4 and 6 nm, respectively. The temperature of the sample was checked with an accuracy of ±0.1°C, using a thermistor thermometer. The anisotropy coefficient (r) was calculated according to Shinitzky and Barenholz [22] from the equation:

$$r = \frac{I_{\parallel} - I_{\perp} G}{I_{\parallel} + 2 I_{\perp} G}$$

where $I_{\parallel}$ and $I_{\perp}$ are the intensities of the light emitted with its polarization plane parallel ($\parallel$) and perpendicular ($\perp$) to that of exciting beam. The grating correction factor for the optical system (G) is given by the ratio of vertically to the horizontally polarized emission components, when the excitation light is polarized in the horizontal plane [23]. Blanks were always recorded without added probes to correct for light scattering, which was always very low in our assay conditions.

The degree of fluorescence anisotropy of DPH and DPH-PA reflects rotational diffusion of the probes, which depends on the relative mobility of phospholipid acyl chains, i.e. on the degree of bilayer fluidity [24–26]. Therefore, a high degree of anisotropy reflects a limited rotational diffusion of the probes and, consequently, represents a high structural order or low membrane fluidity, and vice-versa. Only lipid motion affects the probe anisotropy, not the tumbling of membrane vesicles which is very slow as compared with molecular motion [24].

2.2.4. DSC studies

Multilamellar vesicles for DSC were prepared as described above. The lipid concentration was nominally 666 μM in all the samples examined. Insecticides were added from concentrated ethanolic solutions to give the insecticide/lipid molar ratios indicated in the figures. The mixtures (lipid plus insecticide) were allowed to equilibrate for 18–20 h, and then centrifuged at 45 000×g, for 45 min, at 4°C. The wet pellets were hermetically sealed into aluminum pans and placed in a Perkin-Elmer Pyris 1 differential scanning calorimeter. The DSC measurements were performed at a scan rate of 5°C/min, over the temperature range from 20 to 60°C. This rate yielded thermograms essentially identical to those obtained at 1°C/min, meaning that thermodynamic equilibrium is maintained at scanning rates of 5°C/min. To check the reproducibility of the results, three different samples were measured for each insecticide/lipid ratio. For each sample, three heating and two cooling scans were recorded. Cooling scans yielded thermograms very similar to the heating scans, but, and accordingly to the literature [27], the transitions in cooling curves are shifted, by about 1°C, to lower temperatures. Therefore, due to the supercooling phenomenon, accurate thermotropic transitions are evaluated from heating curves. Therefore, heating scans have been used in the present work.

To determine the total phospholipid content of the samples, the aluminum pans were opened and the samples dissolved in chloroform/methanol (5/1) mixtures. Then, phospholipids were quantified by measuring inorganic phosphate [28] released after hydrolysis of the extracts at 180°C in 70% $HClO_4$ [29].

3. Results

3.1. Fluorescence anisotropy studies

Fluorescence anisotropies of DPH, a probe buried in the bilayer core [22], and of DPH-PA, a probe anchored in the bilayer surface by its charged propionic group [30], provide the relative perturbations

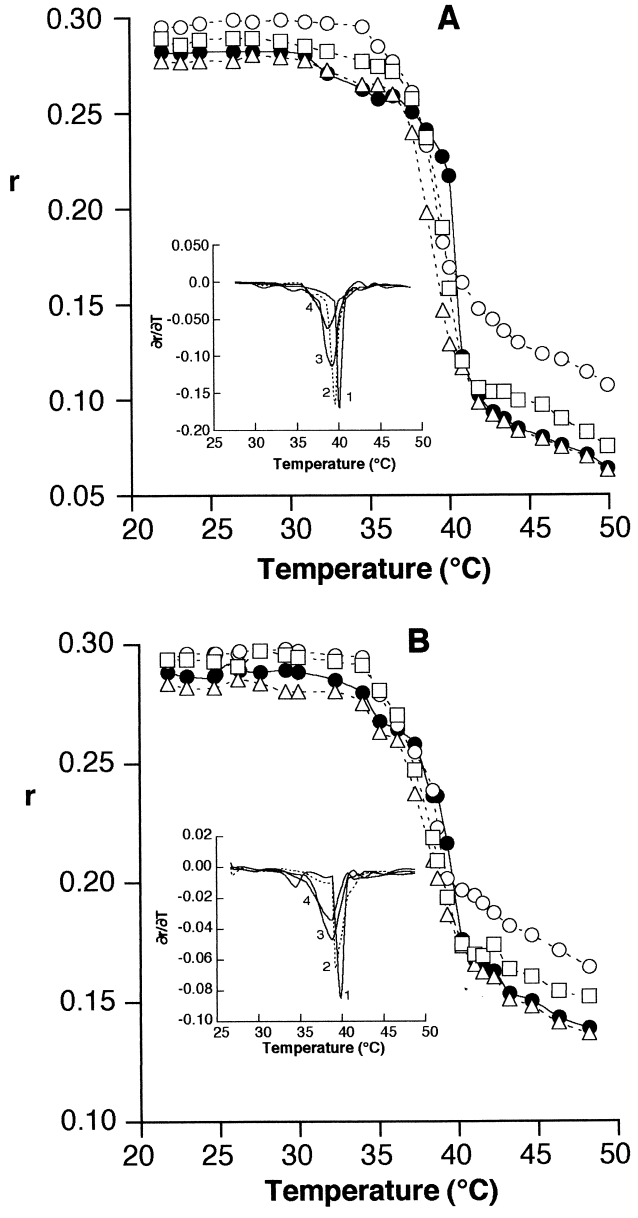

Fig. 2. Fluorescence anisotropy (r) provided by DPH (A) and DPH-PA (B), in DPPC bilayers, as a function of temperature, in the absence (solid symbols and lines) and presence (open symbols and dotted lines) of 50 μM malathion (△), methylparathion (□) and parathion (○). Insets represent the first derivatives of anisotropy data of the main plots. The derivative curves 1, 2, 3 and 4 correspond to the main curves with the symbols ●, △, □ and ○, respectively. The standard deviations (S.D.) of the average of three independent experiments, for each temperature, are too small to be displayed by error bars.

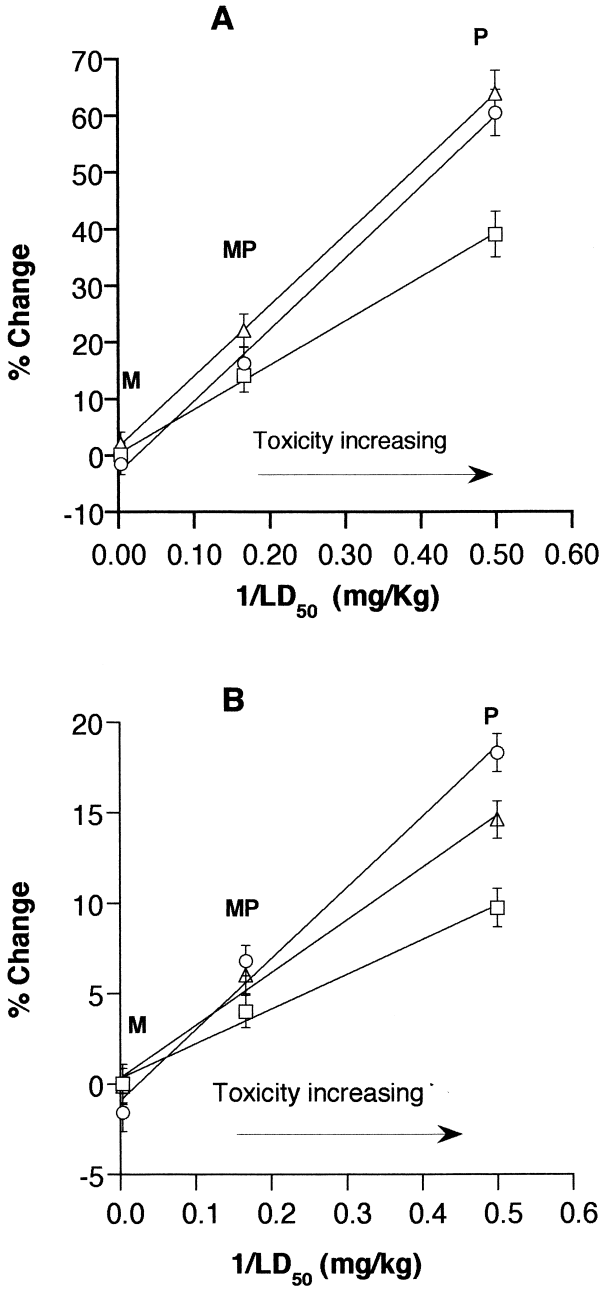

Fig. 3. Fluorescence anisotropy changes, represented as % of control, induced by malathion (M), methylparathion (MP) and parathion (P) in fluid membranes of DPPC (○), mitochondria (△) and sarcoplasmic reticulum (□), as a function of the LD_{50} of the above insecticides to mammals. A and B represent the results obtained with DPH and DPH-PA, respectively. Fluorescence anisotropy values are the means ± S.D. of at least three independent experiments. Fluorescence anisotropy values for mitochondria and sarcoplasmic reticulum were taken from [26–28].

induced by the organophosphorus compounds across the DPPC bilayer thickness. Thus, the effects promoted by 50 μM of malathion, methylparathion and parathion on the thermotropic profile of DPPC are displayed in Fig. 2. The insecticides under study broaden the transition profile and shift the phase transition midpoint (T_m) to lower temperature values. These effects provided by both probes are more apparent for parathion and less apparent for malathion. Methylparathion has intermediate effects. Therefore, the order of the effects correlates reasonably with their toxicity to mammals [1]. Additionally, methylparathion and parathion increase the fluorescence anisotropy of DPPC bilayers, in gel and fluid phase, but mainly in fluid phase (Fig. 2). Again the effects of parathion are stronger than those of methylparathion. Malathion has almost no effect. The ordering effects, which are more apparent when detected with DPH, are consistent with previous data obtained in other models and in several fluid native membranes [31–33]. Considering the fluid phase of DPPC, where the ordering effects are more apparent and representing the fluorescence anisotropy changes versus the toxicity of the insecticides to mammals (represented by LD_{50}), data of Fig. 3 are obtained. Also, in the same figure are represented fluorescence anisotropy changes induced by the insecticides under study in fluid native membranes [31–33], as a function of toxicity to mammals. Either in models or in native membranes it is apparent that parathion, the most toxic insecticide, has the strongest effect, whereas malathion, the less toxic, has the lowest effect; methylparathion, with intermediate toxicity, exerts also intermediate effects.

3.2. DSC

The effects of malathion, methylparathion and parathion on the thermotropic profiles of DPPC were also searched by DSC (Fig. 4). The thermotropic pretransition of DPPC is strongly affected by low amounts of insecticide, and can no longer be detected for parathion/lipid molar ratio of 1/20 or for methylparathion or malathion/lipid molar ratio of 1/7, as shown in Fig. 4. In agreement with fluorescence anisotropy data, the main transition peak broadens and shifts to lower temperature values, an effect that depends on insecticide concentration.

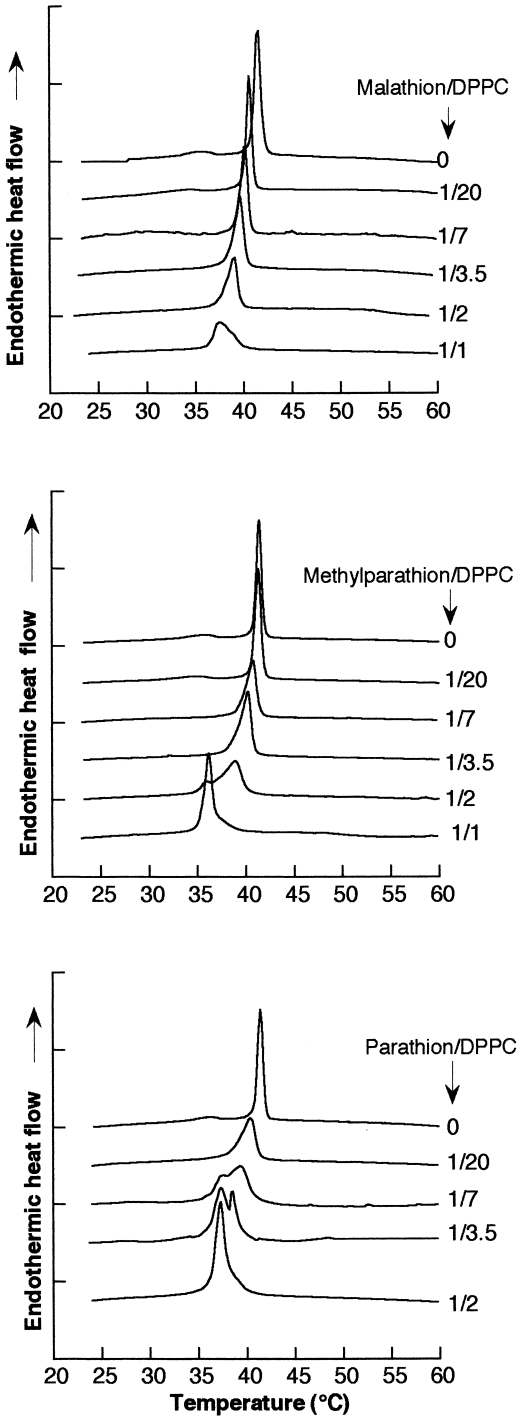

Fig. 4. DSC thermograms for mixtures of insecticide/DPPC. The insecticide/phospholipid molar ratios are indicated on the curves. The DSC profiles correspond to heating scans.

Conversely to malathion, methylparathion and parathion, at insecticide/lipid molar ratios of 1/2 and 1/7, respectively, induce a shoulder in the main peak, in the low temperature side, suggesting coexistence of different phases. At higher insecticide/lipid molar ratios, i.e. 1/1 for methylparathion and 1/2 for parathion, the transition is again sharp and displaced to the position of the previous shoulder, whereas the main peak completely disappears. Therefore, only one peak, with a T_m of about 36°C or 37.4°C for mixtures of methylparathion/DPPC or parathion/DPPC, respectively, is obtained (Fig. 4).

The calorimetric enthalpies of the thermotropic events described in Fig. 4 are depicted in Fig. 5. Concentrations of malathion up to 0.5 insecticide/lipid molar ratio decrease ΔH, but higher insecticide/lipid molar fractions have a limited effect on ΔH. Conversely to malathion, methylparathion and parathion increase ΔH up to a molar ratio of 0.5. Further increase of the ratio has a limited effect on ΔH. These results suggest different interactions between malathion and lipids as compared with methylparathion and parathion.

The above insecticide concentrations were used on the basis of the following findings. LD_{50} values (determined in mammmals, oral administration) for parathion, methylparathion and malathion are 2.0, 6.01 and 290 mg/kg, respectively [34]. Considering a total fluid volume of 58 ml/kg [35], the lethal serum concentrations estimated for parathion, methylparathion and malathion are 118, 394 and 15 100 μM, respectively. Therefore, the concentration of 50 μM, used in fluorescence studies, is far from the lethal doses for these compounds. In DSC studies, the bulk insecticide concentration varied between 0 and 666 μM for methylparathion and malathion and between 0 and 333 μM for parathion. Therefore, for parathion and methylparathion the studies were extended above the lethal doses. However, the second peak in DPPC thermograms appears at 333 μM for methylparathion and at 95 μM for parathion, i.e. at concentrations close to the lethal toxic concentrations in vivo.

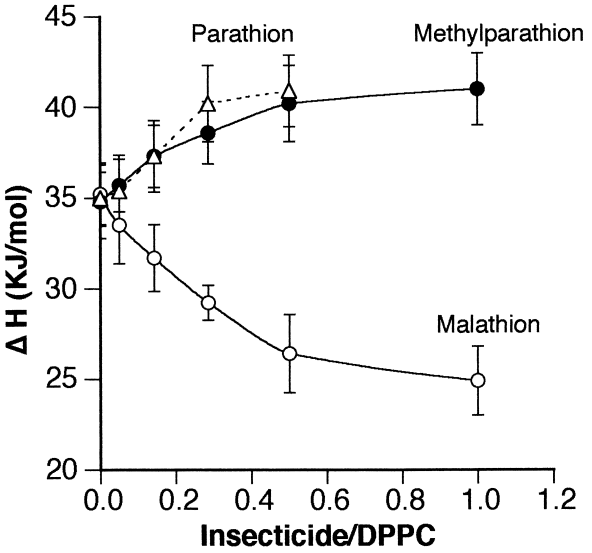

Fig. 5. Enthalpy changes (ΔH, kJ/mol) for the gel to liquid-crystalline phase transition of insecticide/DPPC mixtures, at different insecticide lipid molar ratios. Each point represents the average of at least three independent experiments ± S.D.

4. Discussion

The effects of malathion, methylparathion and parathion on the physicochemical properties of DPPC dispersions were assessed by fluorescence anisotropy and by DSC. To determine the modifications in lipid packing across the bilayer thickness, fluorescence anisotropies of DPH and DPH-PA were measured. Lateral heterogeneities or domains were searched by DSC. This technique also provides valuable information on the phase transition as well as on the enthalpy of melting of membrane components. Therefore, fluorescence anisotropy and DSC provide complementary information about the biophysical modifications occurring in DPPC bilayers as a result of insecticide interaction. Fluorescence anisotropy indicates that the insecticides under study, at concentrations of 50 μM, corresponding to insecticide/lipid stoichiometry of 1/7, shift the phase transition midpoint to lower temperature values and broaden the phase transition (Fig. 2). These effects suggest, accordingly to Jain and Wu [36], a displacement of the xenobiotics into the vicinity of the first eight carbons of the acyl chains, i.e. in the cooperativity region. This is the region where the rigid rings of cholesterol also locate [37]. Interesting is also the fact that an inverse linear relationship between the partition of parathion and malathion and the molar ratio of cholesterol has been observed [7]. Furthermore, a complete exclusion of the insecticides from

the membrane is observed at 50 mol% cholesterol. On the basis of theoretical and experimental approaches [38,39], the way by which cholesterol affects the physical state of lipids and, consequently, the incorporation of insecticides into the bilayers can be discussed as follows. Fluid phospholipid membranes can exist in a liquid-disordered phase, at very low cholesterol concentrations, a liquid-ordered phase, at high cholesterol concentrations, or the two phases, at intermediate sterol concentrations. Apparently, only the liquid-disordered phase allows the incorporation and interaction of this type of insecticides, but the liquid-ordered phase, induced by the packing of cholesterol with lipids in the cooperativity region [37], prevents insecticide incorporation and interaction. Therefore, high cholesterol concentrations increase the density of lipid bilayers in such a way that the free volume for insecticide incorporation and interaction decreases as a consequence of a membrane localization similar to that of sterol.

Conversely to malathion, methylparathion and parathion, at insecticide/lipid molar ratios of 1/2 and 1/7, respectively, induce a shoulder in the main peak, in the low temperature side (Fig. 4). Therefore, at these ratios, the insecticides are non-randomly distributed in the membrane plane. According to Jain and Wu [36], a compound that promotes a new phase transition specifically interacts with the polar headgroups of the phospholipids. Therefore, methylparathion and parathion are probably positioned in the membrane with their polar moieties intercalated between the polar groups of the phospholipids and their hydrophobic moieties buried in the cooperativity region. This location facilitates hydrogen bonding or dipole–dipole interactions between the nitrophenylphosphorothioate group of the insecticides and the headgroups of phospholipids with a consequent decrease in the headgroup spacing. Therefore, a condensing effect is expected to occur, particularly in the fluid phase, extending through the bilayer thickness, as probed by DPH and DPH-PA (Fig. 2). The stronger effect promoted by parathion relatively to methylparathion is certainly related to the chemical structure. The two additional methylene groups of parathion render it more hydrophobic and higher partition is expected. Accordingly, stronger effects are induced by parathion since its core structure is identical to that of methylparathion (Fig. 1). Conversely to methylparathion and parathion, malathion has almost no effect either in the gel or the fluid phase, which may reflect its low partition, since its maximum in DPPC bilayers is 244, whereas the maximal partition for parathion is 1050 [7]. On the other hand, the molecular geometry of malathion is more similar to the molecular geometry of individual phospholipids and, consequently, a good accommodation and miscibility is expected without extensive perturbation of the membrane packing and organization, as compared with the other insecticides (Fig. 2). The results of Fig. 4 also indicate limited perturbation of membrane biophysical properties in mixtures of malathion/DPPC as compared with perturbations expected by methylparathion or parathion/DPPC. On the other hand, a decrease in ΔH for mixtures of malathion/DPPC (Fig. 5) reflects decreasing of van der Waals interactions and indicates that lipid chain packing is different from that of methylparathion or parathion/DPPC mixtures, where an increasing of ΔH is observed. Also, ethylazinphos, another organophosphorus insecticide, induces a new transition in DPPC thermograms [40] and increases ΔH. Furthermore, additional work with this insecticide (article submitted) supports the phase heterogeneity. Ethylazinphos increases the passive proton permeability of lipid bilayers reconstituted with DPPC and mitochondrial lipids. A significant enhancement of proton permeability was detected at insecticide/lipid molar ratios identical to those inducing phase separation in the plane of DPPC bilayers, as revealed by DSC. The organochlorine insecticides of the cyclodiene family α- and β-endosulfan also affect the thermotropic properties of DPPC bilayers. Conversely to β-endosulfan, α-endosulfan, with higher toxicity, promotes a new peak in the thermograms [41] and increases the proton permeability in DPPC and mitochondrial membranes (results to be published). Lindane, another organochlorine insecticide, also creates lateral heterogeneities in lipid membranes [42]. However, this effect does not promote increase in the permeability but the opposite due to a sealing effect of the interfaces by the insecticide [43]. Other drugs [44–47] with the ability to increase the dynamic membrane heterogeneity will also compromise membrane permeability. Moreover, the existence of discontinuous regions not percolated [48] in a continuous membrane phase would difficult certain

membrane phenomena, e.g. the electron delivery in mitochondrial inner membrane. Such a situation has previously been postulated in studies with ethylazinphos and mitochondrial bioenergetics (article submitted).

It must be stressed that data obtained with model DPPC membranes are useful to characterize general physical perturbations induced in lipid bilayers by xenobiotic compounds. Although the data remain valid they cannot be directly extrapolated to complex native biomembranes with a variety of lipid species, i.e. very heterogeneous structures impossible to characterize with the available physical techniques. Although representative, models are not identical to real biomembranes.

The overall studies carried out by fluorescence anisotropy and DSC report effects of the organophosphorus insecticides in different membrane phases, the effectiveness following the sequence: parathion > methylparathion ≫ malathion. This sequence of effects in lipid physicochemical properties correlates with toxicity to mammals [1]. As has been pointed out, lipid bilayer structure and dynamics are crucial for membrane functionality [17–20]. However, it is unclear which lipid membrane properties are critical for a given membrane function [18,49]. Therefore, there is a need for a systematic study on lipid organization and structure and in certain membrane functions in order to establish a coupling between both levels. These studies would provide novel insights to the understanding of the molecular mechanisms of insecticide toxicity.

Acknowledgements

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References

[1] R. Metcalf, The chemistry and biology of pesticides, in: R. White-Stevens (Ed.), Pesticides in the Environment, Marcel Dekker, New York, 1971, pp. 67–124.
[2] G.W. Ware, Pesticides, Theory and Applications, W.H. Freeman and Company, San Francisco, CA, 1983, pp. 35–67.
[3] M. Eto, Organophosphorus Pesticides: Organic and Biological Chemistry, CRC Press, Cleveland, OH, 1974, pp. 123–133.
[4] S.A. Kardos, L.G. Sultatos, Interactions of the organophosphorus paraoxon and methylparaoxon with mouse acetylcholinesterase, Toxicol. Sci. 58 (2000) 118–126.
[5] H. Ohkawa, Stereoselectivity of organophosphorus insecticides, in: J.R. Coats (Ed.), Insecticide Mode of Action, Academic Press, London, 1982, pp. 163–185.
[6] L.S. Volpe, T.M. Biagioni, J.K. Marquis, In vitro modulation of bovine caudate muscarinic receptor number by organophosphorus and carbamates, Toxicol. Appl. Pharmacol. 78 (1985) 226–234.
[7] M.C. Antunes-Madeira, V.M.C. Madeira, Membrane partitioning of organophosphorus and organochlorine insecticides and its implications for mechanisms of toxicity, Pestic. Sci. 26 (1989) 167–179.
[8] R.A. Videira, M.C. Antunes-Madeira, V.M.C. Madeira, Interaction of ethylazinphos with the physical organization of model and native membranes, Biochim. Biophys. Acta 128 (1996) 65–72.
[9] M.C. Antunes-Madeira, V.M.C. Madeira, Interaction of insecticides with lipid membranes, Biochim. Biophys. Acta 550 (1979) 384–392.
[10] M.C. Antunes-Madeira, A.P. Carvalho, V.M.C. Madeira, Interaction of insecticides with erythrocyte membranes, Pestic. Biochem. Physiol. 15 (1981) 79–89.
[11] M.C. Antunes-Madeira, V.M.C. Madeira, Interaction of insecticides with Ca^{2+}-pump activity of sarcoplasmic reticulum, Pestic. Biochem. Physiol. 17 (1982) 185–190.
[12] A.J.M. Moreno, V.M.C. Madeira, Interference of parathion with mitochondrial bioenergetics, Biochim. Biophys. Acta 1015 (1990) 166–174.
[13] W.R. Brown, R.P. Sharma, Synaptosomal adenosine triphosphatase (ATPase) inhibition by organophosphates, Experientia 32 (1976) 1540–1542.
[14] D. Sitkiewicz, M. Skonieczna, K. Krzywicka, E. Dziedzia, K. Staniszewska, W. Bicz, Effect of organophosphorus insecticides on the oxidative processes in rat brain synaptosomes, J. Neurochem. 34 (1980) 619–626.
[15] B.A. Mobley, R.C. Beesley, Y.S. Reddy, J.L. Johnson, Effects of organophosphorus agents on sarcoplasmic reticulum in skinned skeletal muscle fibers, Toxicol. Appl. Pharmacol. 94 (1988) 407–413.
[16] J.M. Swann, T.W. Schultz, J.R. Kennedy, The effects of organophosphorus insecticides dursban and lorsban on the ciliated epithelium of the frog palate in vitro, Arch. Environ. Contam. Toxicol. 30 (1996) 188–194.
[17] A.G. Lee, Functional properties of biological membranes: a physical-chemical approach, Prog. Biophys. Mol. Biol. 29 (1975) 5–56.
[18] J.F. Tocanne, L. Cézanne, A. Lopez, B. Piknova, V. Schram, J.F. Tournier, M. Welby, Lipid domains and lipid/protein interactions in biological membranes, Chem. Phys. Lipids 73 (1994) 139–158.
[19] J. Sikkema, J.A.M. De Bont, B. Poolman, Mechanisms of

membrane toxicity of hydrocarbons, Microbiol. Rev. 59 (1995) 201–222.
[20] O.G. Mouritsen, K. Jørgensen, A new look at lipid-membrane structure in relation to drug research, Pharm. Res. 15 (1998) 1507–1509.
[21] M.C. Antunes-Madeira, V.M.C. Madeira, Membrane fluidity as affected by the insecticide lindane, Biochim. Biophys. Acta 982 (1989) 161–166.
[22] M. Shinitzky, Y. Barenholz, Fluidity parameters of lipid regions determined by fluorescence polarization, Biochim. Biophys. Acta 515 (1978) 367–394.
[23] B.J. Litman, Y. Barenholz, Fluorescent probe: diphenylhexatriene, Methods Enzymol. 81 (1982) 678–685.
[24] K. Kinosita, S. Kawato, A. Ikegani, A theory of fluorescence polarization decay in membrane, Biophys. J. 20 (1977) 289–305.
[25] J.R. Lakowicz, F.G. Prendergast, Detection of hindered rotations of 1,6-diphenyl-1,3,5-hexatriene in lipid bilayers by differential polarization phasefluorometry, Biophys. J. 24 (1978) 213–231.
[26] B.R. Lentz, Membrane 'fluidity' as detected by diphenylhexatriene probes, Chem. Phys. Lipids 50 (1989) 171–190.
[27] P.W.M. Van Dijck, B. De Kruijff, L.L.M. Van Deenen, J. De Gier, R.A. Demel, The preference of cholesterol for phosphatidylcholine in mixed phosphatidylcholine–phosphatidylethanolamine bilayers, Biochim. Biophys. Acta 455 (1976) 576–587.
[28] G.R. Bartlett, Phosphorus assay in column chromatography, J. Biol. Chem. 234 (1959) 466–468.
[29] C.J.F. Böttcher, C.M. Van Gent, C. Pires, A rapid and sensitive sub-micro phosphorus determination, Anal. Chim. Acta 24 (1961) 203–204.
[30] P.J. Trotter, J. Storch, 3-[*p*-(6-phenyl)-1,3,5-hexatrienyl] phenylpropionic acid (PA-DPH): characterization as a fluorescent membrane probe and binding to fatty acid binding proteins, Biochim. Biophys. Acta 982 (1989) 131–139.
[31] M.C. Antunes-Madeira, R.A. Videira, V.M.C. Madeira, Effects of parathion on membrane organization and its implications for the mechanisms of toxicity, Biochim. Biophys. Acta 1190 (1994) 149–154.
[32] R.A. Videira, L.P.S. Peça, M.C. Antunes-Madeira, V.M.C. Madeira, Effects of malathion on membrane fluidity and its implications for the mechanisms of toxicity, Med. Sci. Res. 22 (1994) 551–553.
[33] V.I.C.F. Lopes, M.C. Antunes-Madeira, V.M.C. Madeira, Effects of methylparathion on membrane fluidity and its implications for the mechanisms of toxicity, Toxicol. Vitro 11 (1997) 337–345.
[34] RTECS, Registry of Toxic Effects of Chemical Substances, 1997, NIOSH, US Department of Health and Human Services, Cincinnati, OH.
[35] M.A. Hollinger, Tables of toxicological importance, in: M.J. Derelako, M.A. Hollinger (Eds.), Handbook of Toxicology, CRC Press, Inc., Boca Raton, FL, 1995, p. 804.
[36] M.K. Jain, N.M. Wu, Effect of small molecules on the dipalmitoyl lecithin liposomal bilayer: III phase transition in lipid bilayer, J. Membr. Biol. 34 (1977) 157–201.
[37] D.M. Engelman, J.E. Rothman, The planar organization of lecithin-cholesterol bilayers, J. Biol. Chem. 247 (1972) 3694–3697.
[38] J.H. Ipsen, G. Karlström, O.G. Mouritsen, H. Wennerström, M.J. Zuckermann, Phase equilibria in the phosphatidylcholine-cholesterol system, Biochim. Biophys. Acta 905 (1987) 162–172.
[39] M.R. Vist, J.H. Davis, Phase equilibria of cholesterol/dipalmitoylphosphatidylcholine mixtures: ^{2}H nuclear magnetic resonance and differential scanning calorimetry, Biochemistry 29 (1990) 415–464.
[40] R.A. Videira, M.C. Antunes-Madeira, V.M.C. Madeira, Biophysical perturbations induced by ethylazinphos in lipid membranes, Chem. Phys. Lipids 97 (1999) 139–153.
[41] R.A. Videira, M.C. Antunes-Madeira, V.M.C. Madeira, Perturbations induced by α- and β-endosulfan in lipid membranes: a DSC and fluorescence polarization study, Biochim. Biophys. Acta 1419 (1999) 151–153.
[42] M.C. Sabra, K. Jørgensen, O.G. Mouritsen, Calorimetric and theoretical studies of the effects of lindane on lipid bilayers of different acyl chain length, Biochim. Biophys. Acta 1233 (1995) 89–104.
[43] M.C. Sabra, K. Jørgensen, Lindane suppresses the lipid-bilayer permeability in the main transition region, Biochim. Biophys. Acta 1282 (1996) 85–92.
[44] T. Mavromoustakos, D.P. Yang, A. Makriyannis, Effects of anesthetic steroid alphaxalone and its inactive Δ^{16}-analog on the thermotropic properties of membrane bilayers. A model for membrane perturbation, Biochim. Biophys. Acta 1239 (1995) 257–264.
[45] K. Jørgensen, J.H. Ipsen, O.G. Mouritsen, M.J. Zuckermann, The effect of anaesthetics on the dynamic heterogeneity of lipid membranes, Chem. Phys. Lipids 65 (1993) 205–216.
[46] J. Zeng, K.E. Smith, P.L.G. Chong, Effects of alcohol-induced lipid interdigitation on proton permeability in L-α-dipalmitoylphosphatidylcholine vesicles, Biophys. J. 65 (1993) 1404–1414.
[47] T. Söderlund, J.Y.A. Lehtonen, P.K.J. Kinnunen, Interactions of cyclosporin A with phospholipid membranes: effects of cholesterol, Mol. Pharmacol. 55 (1999) 32–38.
[48] P.F.F. Almeida, W.L.C. Vaz, T.E. Thompson, Lateral diffusion and percolation in two-phase, two-component lipid bilayers. Topology of the solid-phase domains in-plane and across the lipid bilayer, Biochemistry 31 (1992) 7198–7210.
[49] E.E. Williams, Membrane lipids: what membrane physical properties are conserved during physicochemically-induced membrane restructuring?, Am. Zool. 38 (1998) 280–290.